CLAIMS

Amendments to the Claims

1. (Original) A method of identifying a compound that alters a biological persistence of a Clostridial toxin, the method comprising performing a test localization assay having the steps of:

   (a) contacting a cell that comprises a Clostridial toxin light chain with a test compound,

   (b) observing a localization pattern of the light chain in the cell following contacting the cell with the test compound,

   (c) comparing the observed localization pattern to a localization pattern of a light chain in a cell in an absence of the test compound, and

   (d) identifying a test compound that alters a biological persistence of a Clostridial toxin by determining a change in the localization pattern of the light chain in the cell following contacting the cell with the test compound.

2. (Original) The method of claim 1 wherein the Clostridial toxin is a botulinum toxin type A.

3. (Original) The method of claim 2 wherein the biological persistence of the botulinum toxin type A is reduced.

4. (Original) The method of claim 3 wherein the localization pattern of a light chain of botulinum toxin type A in a cell in the presence of the test compound is less localized to the plasma membrane than the localization pattern of a light chain in a cell in an absence of the test compound.

5. (Original) The method of claim 1 further comprising performing a negative control localization assay that comprises the steps of:

(a) contacting a cell that comprises the light chain with a localization assay negative control compound; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the localization assay negative control compound compared to the localization pattern of the light chain in the cell in the absence of the localization assay negative control compound,

wherein a change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay negative control compound indicates that the test localization assay results are inconclusive.

6. (Original) The method of claim 1 further comprising performing a positive control localization assay that comprises the steps of:

(a) contacting a cell that comprises the light chain with a localization assay positive control compound, wherein the localization assay positive control compound is known to change the localization pattern of the light chain in a cell when contacted with a cell that comprises the toxin; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the localization assay positive control compound compared to the localization pattern of the light chain in the cell in the absence of the localization assay positive control compound,

wherein an absence of change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay positive control compound indicates that the test localization assay results are inconclusive.

7. (Original) The method of claim 1 comprising multiple test localization assays wherein individual test assays are performed using different concentrations of test compound.

8. (Original) The method of claim 1 comprising performing at least a duplicate test localization assays.

9. (Original) The method of claim 1 wherein the cell is selected from the group consisting of: Neuro-2A cells, PC12 cells, SHSY-5Y cells, HIT-T15 cells, HeLa cells, HEK293 cells, and primary and established neuronal culture cells from spinal cord, cortex, hippocampus and dorsal root ganglion.

10. (Original) The method of claim 1 wherein the cell comprises a gene that encodes the light chain, which is expressed to produce the light chain in the cell.

11. (Original) The method of claim 1 wherein the toxin is contacted with the cell in an amount effective to be taken up by the cell and produce an identifiable localization pattern of the light chain in the cell.

12. (Original) The method of claim 1 wherein the light chain is labeled.

13. (Original) The method of claim 1 wherein the light chain is labeled with a radio-active isotope or a fluorescent marker.

14. (Original) The method of claim 1 wherein the light chain is expressed as a fusion protein comprising a light chain fused with a fluorescent marker.

15. (Original) The method of claim 1 wherein the localization pattern is determined using microscopic techniques that allow for the analysis of changes in subcellular localization, including confocal microscopic systems.

16. (Original) The method of claim 1 further comprising a test enzymatic assay that comprises:

(a) contacting a sample containing the light chain with an enzymatic substrate of the light chain in the presence of the test compound; and

(b) determining whether the substrate is processed by the light chain into enzymatic product;

wherein the absence of processing of the enzymatic substrate into enzymatic product indicates that the test compound inhibits enzymatic activity, and the enhancement of processing of the enzymatic substrate into enzymatic product indicates that the test compound enhances enzymatic activity.

17. (Original) The method of claim 16 further comprising performing a negative control enzymatic assay that comprises the steps of:

(a) contacting a sample that comprises the light chain with an enzymatic substrate in the presence of an enzymatic assay negative control compound or no added compound; and

(b) determining whether the enzymatic substrate is processed by the light chain into enzymatic product;

wherein the absence of processing of the enzymatic substrate into enzymatic product indicates that test enzymatic assay results are inclusive.

18. (Original) The method of claim 16 further comprising performing a positive control enzymatic assay that comprises the steps of:

(a) contacting a sample that comprises the light chain with an enzymatic substrate in the presence of an enzymatic assay positive control compound; and

(b) determining whether the enzymatic substrate is processed by the light chain into enzymatic product;

wherein processing of the enzymatic substrate into enzymatic product indicates that test enzymatic assay results are inclusive.

19. (Original) The method of claim 16 comprising multiple test enzymatic assays wherein individual enzymatic test assays are performed using different concentrations of test compound.

20. (Original) The method of claim 16 comprising performing at least duplicate test enzymatic assays.

21. (Original) The method of claim 16 wherein the light chain is from Botulinum types A, B, C_1, D, E, F or G or Tetanus toxin, and the enzymatic substrate is a synaptosomal associated protein, SNAP25, VAMP, cellubrevin, syntaxin or fragments, peptides, peptidomimetics, or mixtures thereof.

22. (Original) The method of claim 16 wherein the processing of it into enzymatic product is determined by Western blot, ELISA assay, GFP-SNAP assay, FRET assay, or a combination of said assays, using an antibody that specifically binds to uncleaved enzymatic substrate and/or enzymatic products.

23. (Original) A method of identifying a compound that inhibits the enzymatic activity of Clostridial toxin comprising a test enzymatic assay that comprises:

(a) contacting a sample containing a light chain of a Clostridial toxin with an enzymatic substrate of the light chain in the presence of the test compound; and

(b) determining whether the substrate is processed by the light chain into enzymatic product;

wherein the absence of processing of the enzymatic substrate into enzymatic product indicates that the test compound inhibits enzymatic activity, and the enhancement of

processing of the enzymatic substrate into enzymatic product indicates that the test compound enhances enzymatic activity.

24. (Original) The method of claim 23 wherein the Clostridium toxin is produced by Clostridium beratti, Clostridium butyricum, Clostridium tetani or Clostridium botulinum.

25. (Original) The method of claim 1 wherein Clostridial toxin is selected from the group consisting of: botulinum toxin types A, B, C_1, D, E, F and G.

26. (Original) The method of claim 23 wherein the Clostridial toxin is botulinum toxin type A.

27. (Original) The method of claim 23 further comprising performing a negative control enzymatic assay that comprises the steps of:

(a) contacting a sample that comprises the light chain with an enzymatic substrate in the presence of an enzymatic assay negative control compound or no added compound; and

(b) determining whether the enzymatic substrate is processed by the light chain into enzymatic product;

wherein the absence of processing of the enzymatic substrate into enzymatic product indicates that test enzymatic assay results are inclusive.

28. (Original) The method of claim 23 further comprising performing a positive control enzymatic assay that comprises the steps of:

(a) contacting a sample that comprises the light chain with an enzymatic substrate in the presence of an enzymatic assay positive control compound; and

(b) determining whether the enzymatic substrate is processed by the light chain into enzymatic product;

wherein processing of the enzymatic substrate into enzymatic product indicates that test enzymatic assay results are inclusive.

29. (Original) The method of claim 23 comprising multiple test enzymatic assays wherein individual enzymatic test assays are performed using different concentrations of test compound.

30. (Original) The method of claim 23 comprising performing at least a duplicate test enzymatic assays.

31. (Original) The method of claim 23 wherein the light chain is from Botulinum types A, B, C_1, D, E, F or G or Tetanus toxin, and the enzymatic substrate is a synaptosomal associated protein, SNAP25, VAMP, cellubrevin, syntaxin or fragments, peptides, peptidomimetics, or mixtures thereof.

32. (Original) The method of claim 23 wherein the processing of it into enzymatic product is determined by Western blot, ELISA assay, GFP-SNAP assay, FRET assay, or a combination of said assays, using an antibody that specifically binds to uncleaved enzymatic substrate and/or enzymatic products.

33. (Original) The method of claim 23 further comprising performing a test localization assay that comprises the steps of:

(a) contacting a cell that comprises a Clostridial toxin light chain with a test compound; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the test compound compared to the localization pattern of the light chain in the cell in the absence of the test compound,

wherein a change in the localization pattern of the light chain in the cell following contacting the cell with the test compound indicates that the test compound alters the biological persistence of the toxin.

34. (Original) The method of claim 33 further comprising performing a negative control localization assay that comprises the steps of:

(a) contacting a cell that comprises the light chain with a localization assay negative control compound; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the localization assay negative control compound compared to the localization pattern of the light chain in the cell in the absence of the localization assay negative control compound,

wherein a change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay negative control compound indicates that the test localization assay results are inconclusive.

35. (Original) The method of claim 33 further comprising performing a positive control localization assay that comprises the steps of:

(a) contacting a cell that comprises the light chain with a localization assay positive control compound, wherein the localization assay positive control compound is known to change the localization pattern of the light chain in a cell when contacted with a cell that comprises the toxin; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the localization assay positive control compound compared to the localization pattern of the light chain in the cell in the absence of the localization assay positive control compound,

wherein an absence of change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay positive control compound indicates that the test localization assay results are inconclusive.

36. (Original) The method of claim 33 comprising multiple test localization assays wherein individual test assays are performed using different concentrations of test compound.

37. (Original) The method of claim 33 comprising performing duplicate test localization assays.

38. (Original) The method of claim 33 wherein the cell is selected from the group consisting of: Neuro-2A cells, PC12 cells, SH-SY5Y cells, HIT-T15 cells, HeLa cells, HEK293 cells, and primary and established neuronal culture cells from spinal cord, cortex, hippocampus and dorsal root ganglion.

39. (Original) The method of claim 33 wherein the cell comprises a gene that encodes the light chain, which is expressed to produce the light chain in the cell.

40. (Original) The method of claim 33 wherein the toxin is contacted with the cell in an amount effective to be taken up by the cell and produce an identifiable localization pattern of the light chain in the cell.

41. (Original) The method of claim 33 wherein the light chain used in the assay is labeled.

42. (Original) The method of claim 33 wherein the light chain used in the assay is labeled with a radio-active isotope or a fluorescent marker.

43. (Original) The method of claim 33 wherein the light chain used in the assay is expressed as a fusion protein comprising a light chain fused with a fluorescent marker.

44. (Original) The method of claim 33 wherein the localization pattern is determined using microscopic techniques that allow for analysis of changes in subcellular localization, including confocal microscopy.

45. (Original) A method of identifying a compound that inhibits the biological persistence of a Clostridial toxin comprising performing a test localization assay that comprises the steps of:

(a) contacting a cell that comprises a Clostridial toxin light chain with a test compound; and

(b) determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the test compound compared to the localization pattern of the light chain in the cell in the absence of the test compound,

wherein a change in the localization pattern of the light chain in the cell following contacting the cell with the test compound indicates that the test compound inhibits the biological persistence of the toxin.

46. (Original) The method of claim 45 wherein the Clostridial toxin is produced by Clostridium beratti, Clostridium butyricum, Clostridium tetani or Clostridium botulinum.

47. (Original) The method of claim 45 wherein Clostridial toxin is selected from the group consisting of: botulinum toxin types A, B, C_1, D, E, F and G.

48. (Original) The method of claim 45 wherein the Clostridial toxin is botulinum toxin type A.

49-55 (Cancelled).